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Clinical evidence for thrombospondin-1 as a relevant suppressor of liver regeneration

To the Editor:

The most significant factor determining morbidity and mortality following hepatectomy is the ability of the remnant liver to regenerate. In this context, Hayashi *et al.* [1] recently reported on a previously unknown role of thrombospondin-1 (TSP-1) in liver regeneration (LR). During tissue remodeling, TSP-1 is released into the pericellular space to regulate cell survival and extracellular matrix remodeling [2]. A major TSP-1 function is the conversion of latent TGF-β_1 (transforming growth factor-beta 1) to its active form [3]. Based on a mouse model of partial hepatectomy, Hayashi *et al.* [1] demonstrated that TSP-1 was rapidly released in response to liver resection and triggered TGF-β_1 activation and signal transduction in hepatocytes as a mechanism to inhibit the proliferative hepatic response [4–5]. Accordingly, knockdown of TSP-1 expression resulted in a significant reduction of TGF-β_1 signaling, which promoted the proliferation of hepatocytes and accelerated LR [1]. Thus, TSP-1 was proposed as an inhibitory element and a potential therapeutic target of LR. We are now able to present the first clinical data, which support the relevance of TSP-1 in LR based on a cohort of metastasized colorectal cancer (mCRC) patients undergoing hepatic resection of liver metastases.

A total of 66 mCRC patients without preexisting liver disease were included. Blood was retrieved before surgery (pre OP) and on day 1 (POD1) or days 5–8 (POD5) after liver resection, and plasma was prepared with an optimized method [6–7]. Samples were assayed by ELISA for human TSP-1 (R&D Systems, Minneapolis, MN, USA) and TGF-β_1 (eBioscience, San Diego, CA, USA). For statistical analysis, non-parametric tests (Mann–Whitney U test, Wilcoxon test and Spearman correlation) and the Fisher exact test were applied.

A significant increase in TSP-1 (Fig. 1A) and TGF-β_1 was observed on POD1 ($p = 0.007$ and $p = 0.006$, respectively), which dropped moderately until POD5 ($p = 0.016$ and $p = 0.397$). Circulating TSP-1 and TGF-β_1 showed a highly significant correlation ($k = 0.736$, $p < 0.001$). To further investigate whether a high postoperative release of TSP-1 or TGF-β_1 was associated with delayed LR, we assessed the incidence of postoperative liver dysfunction (LD), based on the previously defined "50–50 criteria" [8], with a prothrombin time (PT) <50% and a serum bilirubin (SB) level >50 μmol/L (>2.9 mg/dl). Our classification referred to measurements of any day within the first postoperative week to identify patients with delayed postoperative hepatic recovery. TSP-1 concentrations were significantly elevated (Fig. 1B) in patients with LD (median TSP-1 for LD: 98.8 ng/ml; no LD: 44.6 ng/ml, $p = 0.003$). Comparably, circulating TGF-β_1 tended to be increased in individuals with LD (median TGF-β_1 for LD: 2266 pg/ml; no LD: 1726 pg/ml), but the difference was not statistically significant ($p = 0.102$). Of note, our analyses were restricted to total TGF-β_1 in patient blood since activated TGF-β_1 was below ELISA detection limit. This may explain the lack of association between TGF-β_1 levels and postoperative LD.

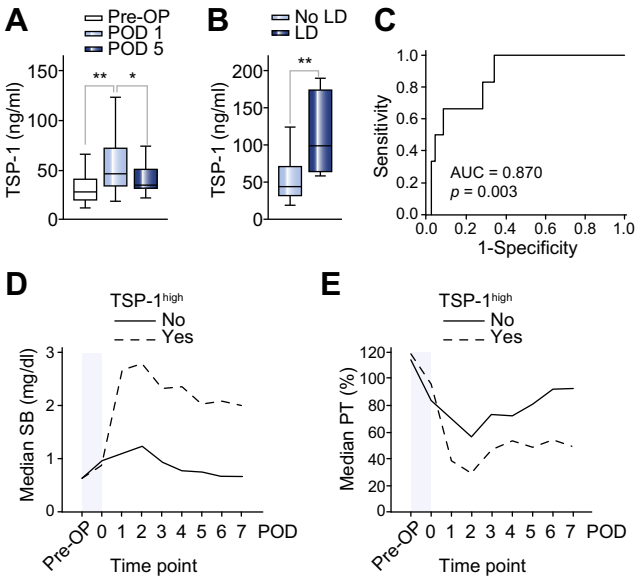

Fig. 1. Association of plasma TSP-1 with parameters of postoperative liver regeneration. (A) Plasma levels of TSP-1 were determined in mCRC patients before surgery, on the first postoperative day (POD1) and between POD 5–8. Patients with or without postoperative liver dysfunction (LD) were compared for circulating TSP-1 by (B) boxplot illustration (without outliers and extreme values; $^{*}p < 0.05$; $^{**}p < 0.01$) and by (C) ROC analysis. The perioperative time course of median liver function parameters (D) serum bilirubin (SB) and (E) prothrombin time (PT) was evaluated separately for patients with low (⩽100 ng/ml) and high (>100 ng/ml) circulating plasma TSP-1 on POD1.

To further characterize the potential of TSP-1 to predict postoperative LD, a ROC analysis was performed (Fig. 1C), revealing a significant predictive value of circulating TSP-1 on POD1 (AUC: 0.870; $p = 0.003$). A cut-off level of 100 ng/ml TSP-1 on POD1 was deduced to determine the high-risk group for postoperative LD with 95% specificity. No statistically significant difference in age and sex distribution or the site of primary tumor, type of hepatic resection and preoperative liver function parameters was observed between the TSP-1^{high} individuals (>100 ng/ml TSP-1) and patients with lower circulating TSP-1 levels on POD1.

When biochemical markers of postoperative liver function were compared between these groups, the TSP-1^{high} patients showed a significant elevation in SB (Fig. 1D) and γ-glutamyltransferase ($p < 0.001$; $p = 0.011$). Prothrombin time (Fig. 1E) was found to be significantly decreased in TSP-1^{high} patients ($p < 0.001$). A trend towards elevated alanine and aspartate

aminotransferase levels was found in TSP-1^{high} patients but did not reach statistical significance ($p = 0.125$ and $p = 0.092$).

We further assessed the clinical performance of TSP-1^{high} individuals in terms of morbidity and hospital stay. Only 4 patients suffered from severe morbidity of grade III, IV or V according to Dindo *et al.* [9], which limits the conclusions of the statistical analysis. However, TSP-1 levels were significantly elevated in patients with severe complications ($p = 0.016$) and we found a substantial increase of postoperative severe morbidity from 4% to 33% in the TSP-1^{high} subgroup ($p = 0.053$). With respect to hospital stay, a highly significant correlation of TSP-1 levels on POD1 with days of postoperative hospitalization was observed ($k = 0.488$; $p < 0.001$). In accordance, TSP-1^{high} patients required a doubled median hospitalization time of 16.5 days as compared to 8 days for the remaining patients to recover from hepatectomy ($p = 0.011$).

In conclusion, these are the first clinical data confirming that TSP-1 negatively correlates with liver regeneration in humans, which warrants validation in large-scale studies. TSP-1 may prove a helpful clinical marker to predict LD on the first postoperative day and to identify high-risk patients for potential complications and suitable interventions.

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Conflict of interest

The authors who have taken part in this study declare that they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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